had totally different ERIC-PCR patterns despite identical antibiograms.

Conclusions: ERIC-PCR typing offers a simple, rapid and highly discriminatory method for identification of *A. baumannii* outbreak strains.

Pseudomonas infections – cystic fibrosis

P885 Risk Factors for the Clinical Detection of Imipenem-Resistance in *Pseudomonas aeruginosa*

N. Troillet, M.H. Samore, Y. Carmeli. *Beth Israel Deaconess Medical Center, Boston, USA*

Objectives: To determine risk factors (RFs) for the clinical detection of imipenem (IMP)-resistant *P. aeruginosa* (*Pa*) in hospitalized patients.

Methods: Case-control study using prospectively collected data over a two-year period. Cases were patients with a first *Pa* isolate resistant or intermediate to IMP (MIC < = 8 μg/ml). Controls were patients with a first IMP-susceptible *Pa* isolate (MIC < 8 μg/ml). Univariate analysis of the hypothesized RFs was done by Fisher's exact tests, two-sample Wilcoxon tests, or t-tests, as appropriate. Multivariate logistic regression was used to adjust for confounding.

Results: In univariate analysis the 40 cases were more likely than the 387 controls to have received IMP (Odds ratio = 16.9, $p < 0.0001$) and to be organ transplantated patients (OR = 3.9, $p = 0.008$). No significant difference was found for treatments with other β-lactams or aminoglycosides, for other underlying diseases or the Charlson score (weighted comorbidities), for the sex, the age, the length of the hospital stay, having stayed in ICUs, undergone a surgical procedure or shared room with a case, for prior hospital admissions within a year, transfers from others hospitals or from nursing homes, and for the culture site. Multivariate analysis confirmed the association between IMP therapy and the detection of IMP-resistant *Pa* (OR = 23.2, $p = 0.0004$), and between organ transplant status and the detection of IMP-resistant *Pa* (OR = 3.4, $p = 0.01$).

Conclusions: Treatment with IMP, but not with other β-lactams, is a major RF for the detection of IMP-resistant *P. aeruginosa* in hospitalized patients. No other RF related to the hospital environment was identified.

P886 Predominance of O:11 as well as O:12 Multi-Resistant *Pseudomonas aeruginosa* from Greek Hospitals

P.T. Tassios, V. Gennimata, A.N. Maniatis, C. Fock, N.J. Legakis. *Department of Microbiology, Medical School University of Athens, Athens, Greece*

In collaboration with Hospital Microbiology Departments headed by: J. Douboyias (AHEPA), A. Katrahoura (Metaxa), S. Kitsou (Ag. Olga), C. Koutsia (Voula), K. Bethymouti (Erythros), M. Delopoulos (401), S. Dova (Ag. Savvas), C. Oikonomopoulou (Tzanneio), O. Paniara (Evangelismos), E. Papafrangas (Sismanogleio), E. Papoutsaki (KAT).

Objectives: This study was a first attempt to correlate the phenotypic and genotypic characteristics of Greek nosocomial *Pseudomonas aeruginosa* strains.

Methods: Seventy nine randomly selected nosocomial isolates from eleven Greek hospitals, collected during the period 1994–95, were studied with respect to their antibiotic resistance, serotypes, bacteriocin types and DNA fingerprints, obtained by pulsed field gel electrophoresis (PFGE) of *XbaI*-restricted genomic DNA.

Results: Serotyping and bacteriocin typing yielded 13 and 60 types, respectively, while PFGE was able to discriminate further among phenotypically indistinguishable isolates. Multi-resistant isolates (43%) grouped in serotypes O:12 (41%) and O:11 (26.5%), the former exhibiting greater genetic homogeneity than the latter, with 80% of O:12 strains belonging to PFGE group A, while only 27% of O:11 strains clustered in a common PFGE group, C. Group A was represented in nine out of the eleven participating hospitals.

Conclusions: The genetic homogeneity of the O:12 multi-resistant clone, known to be dominant in Greece as well in other European countries, was confirmed by PFGE, while a second, less genetically homogeneous multi-resistant cluster was found to be characterised by serotype O:11.

P887 Microbiology of Burns in Hospitalized Children

G. Kouppari, A. Zaphiropoulou, M. Tsirepa, N. Xenos, V. Deliyianni. *Microbiol. Laboratory of "P. and A. Kyriakou" Children's Hospital, Athens, Greece*

Objectives: The study of the microbiology of burns in children who required hospitalization.

Methods: Material of this study were samples from burn wounds from 181 children (0–14 years old) who required hospitalization during a two-years period (1/10/93–30/9/95). The isolation and identification of bacteria were performed by conventional methods and the susceptibility testing by Bauer-Kirby method.

Results: The incidence of burns is higher among children 1–2 years old. In 23.7% of the samples one species of microorganism was isolated, in 23.7% two species, in 14.4% three, in 8.8% four, in 7.2% five and in 15.5% $\geq$6 species. In 6.6% of the samples no microorganism was isolated. The most frequently isolated bacteria were *Staphylococcus co(−)* (CNS) (34.6%) followed by *Streptococcus viridans* (17.8%), *Enterobacteriaceae* (13.6%), *Staphylococcus aureus* (8%), *Pseudomonas spp* (7.5%), *Acinetobacter spp* (6.1%) and miscellaneous microorganisms in smaller numbers. From CNS 76.8% were resistant to penicillin, 28.9% to oxacillin, 45.4% to erythromycin, 6.7% to clindamycin and 0% to vancomycin and rifampicin. The resistance of *S. aureus* was 93.3%, 33.3%, 22.2%, 4.4% and 0% respectively.

Conclusions: Burns are more frequent in 1–2 years old children. The majority of burn wounds are colonized by $\geq$2 microbial species and in 15.5% by $\geq$6 species. The most frequent isolates were CNS, *Streptococci, Enterobacteriaceae, S. aureus* and *Pseudomonas spp.*

P888 Clinical and Paraclinical Investigation of Ciprofloxacin in Burn Patients with Pseudomonas Infection

A. Rastegar lari [1], R. Nikui, S.M. Mousavi [2], S.M. Mousavi [2], R. Rahimi [2]. [1]*Dept. of Microbiology, Iran University of Medical Sciences, Iran,* [2]*Razak Laboratory Co., Tehran, Iran*

Objectives: Pseudomonas aeruginosa is a major cause of infection in hospitalized patients especially in burned. Resistant microorganisms is another complication in these patients. This study was made to determine efficacy of ciprofloxacin against pseudomonas infection in burn patients.

Methods: Thirty seven hospitalized burn patients older than 15 years with burn surface around 10–80 percent of 2nd and 3rd degree burn were participated. Ciprofloxacin was administered (500–750 mg tds) for 7–20 days.

Results: In this study 30 patients had positive culture for pseudomonas. It is found that 90 percent of pseudomonas strains were

sensitive to ciprofloxacin with MIC less than 2 microgram/ml. Sixty percent of isolated strains from burn patients were resistant to amikacin and all were resistant to ceftizoxime, carbenicillin and co-trimoxazol. Three patients (8%) under this therapy died because of more than 50 percent burn surface.

Conclusions: It is concluded that Pseudomonas aeruginosa strains are highly susceptible to ciprofloxacin but its over usage of this drug may induce resistancy and complication in therapy.

P889 Nosocomial *Sphingomonas paucimobilis* Infections

P.R. Hsueh, L.J. Teng, S.W. Ho, K.T. Luh. *National Taiwan University, Taipei, Taiwan*

Objectives: To determine the role of *Sphingomonas paucimobilis* on the nosocomial infection.

Methods: A total of 18 isolates of *S. paucimobilis,* including 11 from clinical specimens of seven patients with nosocomial infection and seven from environmental sources (dialysates, humidifier fluids, and tap water), were studied on the biotypes by API 20NE and Vitek GNI card, the cellular fatty acid composition by gas-liquid chromatography, antimicrobial susceptibility by using the Etest, and random amplified polymorphic DNA (RAPD) patterns generated by arbitrarily primed PCR.

Results: There were 11 biotypes and 11 RAPD patterns identified. Isolates recovered from different patients and different environmental sources possessed different biotypes or RAPD patterns. The identical biotype and RAPD pattern of the two isolates (one each from blood and bile) from a patient with biliary tract infection indicated the invasiveness of the organism from biliary tract into bloodstream. Two patients with intravascular catheter-related bacteremia had repeated blood isolations of this organism with a interval of four days to one month, suggesting the ability of this organism to colonize the catheter and result in recurrent bacteremia. All isolates were susceptible to minocycline, ciprofloxacin, trimethoprim/sulfamethoxazole, ciprofloxacin, and imipenem.

Conclusions: The study on biotypes as well as RAPD patterns of *S. paucimobilis* isolates both may be considered as a valuable tool of epidemiological surveillance of nosocomial infections caused by this organism.

P890 Epidemiological Typing of *Stenotrophomonas Maltophilia* by PCR

M. Mueller-Premru [1], P. Černelč [2]. [1] *Institute of Microbiology, Medical faculty, University of Ljubljana, Slovenia,* [2] *Department of Haematology, University Medical centre, Ljubljana, Slovenia*

Objectives: To perform epidemiological typing of *Stenotrophomonas (S.) maltophilia* from five departments.

Methods: Typing by ERIC-PCR with primer derived from the enterobacterial repetitive intergenic consensus (ERIC) sequences was performed on 11 *S. maltophilia* isolates. Five of them have been isolated from respiratory specimens of patients in haematology department, two in burn, two in nephrology, one in infectious diseases and one in cardiology department. Antibiotic susceptibility testing with cefotaxime (30 μg), gentamicin (10 μg), amikacin (30 μg), piperacillin (100 μg), mezlocillin (75 μg), imipenem (10 μg), trimethoprim/sulphamethoxazole (1.25/23.75 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg) and cefoperazone (30 μg) was performed according to the Kirby-Bauer's disk diffusion method.

Results: Ten PCR patterns were obtained among 11 *S. maltophilia* isolates. The isolates from different departments had different PCR patterns and appeared unrelated. Inside departments the isolates appeared unrelated with the exception of the two isolates in nephrology department. Isolates were resistant to cefotaxime, gentamicin, amikacin, piperacillin, mezlocllin and imipenem. Ten were susceptible to trimethoprim sulphametoxazole, 11 to chloramphenicol, five to ciprofloxacin and two to cefoperazone.

Conclusions: The *S. maltophilia* isolates appeared unrelated among different departments and mostly inside individual departments.

P891 *Pseudomonas aeruginosa* Colonization in CF Patients – Pyocintyping and Typing with Pulse-Field Gel Electrophoresis

F.B. Spencker [1], S. Haupt [2], T. Lietz [2], S. Walter [2], R. Schille [2]. [1] *Institute of Medical Microbiology and Epidemiology of Infection,* [2] *Department of Pediatrics, University of Leipzig, Leipzig, Germany*

Objectives: To determine steadiness and variability of colonization in CF patients over long-term periods and to check the usefulness of pyocintyping and typing with pulse-field gel electrophoresis (PFGE) for comparing strains.

Methods: A total of 513 *Pseudomonas aeruginosa (Ps. ae.)* isolates had been found from 44 patients during the period from April 1994 to April 1996. The sources were mainly sputum and a few deep throat swabs. All strains from colonies dissimilar in macroscopic appearance had been processed separately. For pyocintyping the procedure described by Fyfe (1984) was applied. Typing with PFGE was carried out by contour-clamped homogeneous electric field electrophoresis. Genomic DNA was subjected to rare-cutting restriction enzyme SpeI.

Results: We found 18 different pyocintypes; one further pyocintype was not classifiable. The most frequent pyocintype was No. 3 followed by the types 1 and 5. After typing with PFGE we observed 20 genotypes. There was some correlation between results of pyocintyping and PFGE but no total correspondence.

Conclusions: Typing with PFGE is well suited for detailed investigations of colonization with *Ps. ae.* in CF patients. For conclusive evidence of cross-colonization genotyping techniques are mandatory.

P892 Analysis of Variability of *Pseudomonas aeruginosa* Strains Isolated from Bronchiectasis Patients by Different PCR Assays

I. Pujana [1], L. Gallego [1], G. Martín [2], E. Arrese [1], P. Sanz [1], F. López [1], R. Cisterna [1]. [1] *Dept. of Microbiology, Faculty of Medicine, University of Basque Country, Spain,* [2] *Hospital de Santa Marina, Bilbao, Spain*

Objectives: The objective of the study was to ascertain if it was the same strain of *P. aeruginosa* which reappears, after some time, in the sputum of chronic bronchiectasis patients or whether it was reinfection with a new strain.

Methods: Arbitrarily primed PCR (AP-PCR) and enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) techniques were used to study 35 strains of *P. aeruginosa* collected from the sputum of 11 patients. Genomic DNA was obtained by a lisis with proteinase K and Sodium Dodecyl Sulfate. All the isolates were tested separately with the following primers: a) AP3 (5′-TCACGATGCA-3′) b) ERIC1 (5′-GTGAATCCCCAGGAGCTTACAT-3′). Amplified DNA was separated by gel electrophoresis in 1.5% agarose. Bands were visualized using ethidium bromide staining and photographed on a UV transilluminator and compared for each isolate.

Results: Of the eleven patients, 8 harbored a single dominant strain of *P. aeruginosa,* with an intrapatient similarity pattern range of

85 to 100%. The other 3 patients were infected with mixed bacterial isolates.

Conclusion: In conclusion most of the patients are chronically infected by the same strain. Our results show the fact that AP-PCR and ERIC-PCR techniques can be used efficiently and effectively to differentiate *P. aeruginosa* strains in bronchiectasis patients independently of their phenotypic expression.

P893 Prevalence and Antibiotic Susceptibility Profile of *Haemophilus influenzae* Isolates from Cystic Fibrosis Patients

R. Cantón, N. Mir, S. Valdezate, M.E. Alvarez, C. Calderón, J. Martínez-Beltrán, F. Baquero. *Servicio de Microbiología, Hospital Ramón y Cajal, Madrid, Spain*

Objective: To determine the prevalence of bronchopulmonary *H. influenzae* colonization in cystic fibrosis (CF) patients and the antibiotic susceptibility profile.

Methods: Respiratory samples from 88 CF patients (0.5–41 years) obtained from 1993 to 1996 were studied by a quantitative technique which included bacitracin-chocolate plates. Susceptibility was performed with the agar and microdilution techniques with 5%-chocolate blood and 2%-IsoVitalex MH agar and HTM broth.

Results and Conclusions: 87 *H. influenzae* isolates were recovered from 32 patients (36.3%); 17 of them had a single isolate during this period, and 3 were chronic colonized. Susceptibility profiles expressed as MIC range (μg/ml) were:

Antibiotic	Amp-S $\beta\iota(-)$ (n = 37)	Amp-R $\beta\iota(-)$ (no = 8)	Amp-R $\beta\iota(+)$ (n = 42)
Ampicillin	0.1–1	2–>8	4–>8
Co-amoxiclav	≤0.5/0.2–1/0.5	1/0.5–8/4	≤0.5/0.2–2/1
Cefuroxime	≤0.5–1	1–8	0.5–>8
Cefotaxime	≤0.06–0.1	≤0.06–0.5	≤0.06–0.1
Imipenem	≤0.06–0.5	0.1–0.5	≤0.06–0.5
Chloramphenicol	≤2	≤2	≤2–8
Rifampicin	≤0.2–2	0.5–>2	0.5–>2
Erythromycin	1–4	2–4	1–>4
Azitromycin	≤0.2–4	1	≤0.2–>4
Ciprofloxacin	≤0.01–0.03	≤0.01–32	≤0.01–32

Considering no repeated isolates, ampicillin resistance was 52.5%; 40.0% β-lactamase positive and 12.5% β-lactamase negative. Chloramphenicol resistance were 7.5%.

In 2 chronic colonized patients, the strains were resistant (8–64 μg/ml) or less susceptible (0.5–2 μg/ml) to fluorquinolones.

P894 Molecular Epidemiology of *Pseudomonas aeruginosa (P. a)* Isolated from Cystic-Fibrosis Patients Before and After Transplantation

P. Honderlick[1], D. Grenet[2], M. Stern[2], P. Cahen[1]. [1]*Microbiology, C.M.C Foch, Suresnes, France,* [2]*Pneumology, C.M.C Foch, Suresnes, France*

Pulsed Field Gel Electrophoresis (PFGE) was used to study retrospectively 50 strains of *P. a* from 5 Cystic-Fibrosis patients who benefit of double Lung Transplantation in our hospital. The objectives of the study were multiple:

Searching the emergence of a "single center specific *P. a* strain" supporting the concept of nosocomial acquisition.

Looking after lung transplantation for a cross transmission of *P. a* between patients attending conferences in our cystic-fibrosis center (same area, same physicians...).

Confirming that for a cystic-fibrosis patient the strains implicated in repeated pulmonary infection are relatively homogeneous.

Finally, comparing for an individual patient the DNA Banding Patterns of *P. a* strains before and after transplantation. PFGE was practiced on 12 pre-transplant and 38 post-transplant strains. Isolates were studied using cut restriction Enzyme SpeI. Isolates which differed by no more than 3 restriction fragment positions were considered as subtypes.

No cross contamination was found between PFGE patients, nor center specific strain of *P. a* reveal by a unique genomic fingerprint.

As expected, PFGE appears as a more discriminative method than antibiotype and serotype.

Concerning our 5 patients we found for each one at least one *P. a* persistent strain with the same PFGE pattern before and after transplant proceeding.

Clostridium difficile

P895 Molecular Characterization of a Toxin B-positive, toxin A-negative Strain of *C. difficile*

S.-Y. Chang, K.-P. Song. *National University of Singapore, Republic of Singapore*

Introduction: *Clostridium difficile* is a human pathogen which produces toxins A and B. Toxigenic strains of *C. difficile* (10,463) are the major cause of antibiotic associated pseudomembranous colitis. Although *C. difficile* strains vary significantly in how much of both toxins they produce, a 1:1 ratio is maintained by all strains suggesting that the production of both toxins is coordinately regulated. It was recently found that strain 8864, a naturally occuring strain, only produces toxin B in the absence of toxin A. Based on some preliminary results obtained in other laboratories, it was suggested that a major portion of the toxin A gene, especially those at the 3′-end, is absent.

Objectives: To determine the amount of toxin A gene absent in strain 8864, as compared to strain 10,463.

Methods: Genomic DNA from the two strains were extracted and purified. Digestions using various restriction enzymes were performed so that different DNA fragments were generated. Both dot blots and Southern hybridizations, were done using oligonucleotides that recognize gene sequences in toxin A gene.

Results: The same size bands were hybridized in the Southern blot analysis from the two strains.

Conclusion: The toxin A gene in strain 8864 is the same size as the one in strain 10,463.

P896 *Clostridium difficile* Associated Diarrhea (CDAD) in Heart Transplant (HT) Patients

P. Muñoz, T. Pelaez, J. Palomo, R. Muñoz, M.J. Ruiz-Serrano, E. Bouza. *Hospital "Gregorio Marañón", Madrid, Spain*

Introduction: CDAD is almost unreported in HT patients, despite surgery, prolonged hospital stays and immunosuppression.

Results: From January 1993 to December 1995, 84 patients received one or more HT. Norfloxacin, nystatin, and twice-a-week cotrimoxazole were given for prophylaxis. CDAD was detected in 14 patients (16.6%) by means of plate culture and cytotoxicity assay for *C. difficile* toxin B. Incidence evolved from 8.6% in 1993 to 22% in 1995. CDAD appeared a mean of 51 days after HT (12–144). Mean age was 54.5 years and 79% were men. Patients had been at the hospital for a mean of 64 days before CDAD and they all had a previous or concurrent infection (CMV disease in 57%). Most common manifestations were watery diarrhea (100%), with a mean of 10 daily bowel movements, abdominal pain (29%), fever (14%) rectal